Sensors and Actuators B 247 (2017) 46–52

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Short Communication

A simple pH fluorescent probe based on new fluorophore indolizine for imaging of living cells

Yanqing Ge[a], Aikun Liu[a], Jian Dong[a], Guiyun Duan[a], Xiaoqun Cao[a,*], Fuyou Li[b]

[a] School of Chemical Engineering, Taishan Medical University, Taian, Shandong 271016, PR China
[b] Department of Chemistry and The State Key Laboratory of Molecular Engineering of Polymers and Institute of Biomedicine Science, Fudan University, Shanghai, 200433, PR China

ARTICLE INFO

Article history:
Received 20 January 2017
Received in revised form 23 February 2017
Accepted 24 February 2017
Available online 6 March 2017

Keywords:
Indolizine
Bioimaging
ICT
Fluorescent probe

ABSTRACT

A simple pH fluorescent probe based on new fluorophore indolizine derivative has been synthesized and characterized. This probe responds to acidic pH (pKa = 3.85) with high quantum yield ($\varphi = 0.52$), short response time (within 1 min), high selectivity and sensitivity. The response mechanism of the fluorescent probes relies on ICT changes. The probe was successfully used to monitor intracellular H^+ within RAW 264.7 cells.

1. Introduction

Intracellular pH plays extremely important roles in cellular behaviours and pathological conditions [1–3]. Every organelle has a different pH distribution. For example, lysosomes and endosomes have a local pH range from 4.5 to 6.8 [4–7]; Cytoplasma has a pH range of 6.8–7.4 [8,9]; Mitochondria are active at a slightly basic pH of around 8 [10,11]. The abnormal pH variation will cause many common diseases such as cystic fibrosis, neurodegenerative disorders, Alzheimer's disease and cancer [12–14]. Consequently, monitoring pH changes inside living cells is critical for studying cellular functions and better understanding physiological and pathological processes.

A variety of techniques including electrochemical, nuclear magnetic resonance (NMR) and absorption spectroscopy have been reported to measure pH value [15–17]. Because of the operational simplicity, high sensitivity, and excellent spatial and temporal resolution, fluorescence probe is becoming one of the most powerful tools for monitoring intracellular pH [18].

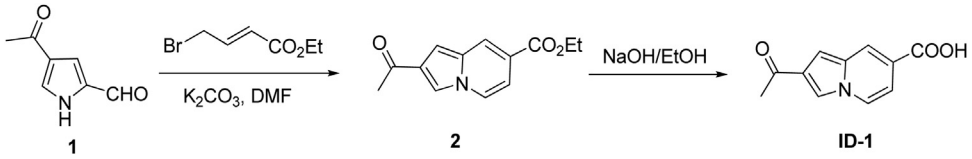
To date, lots of pH fluorescent probes have been designed. These indicators display a desirable pH-dependent fluorescence emission during ionization and deionization processes. However, they also show some drawbacks such as the influence of the excitation power, low fluorescence efficiency and fast photo bleaching rate [19]. Furthermore, although numerous pH-dependent fluorescent probes with near neutral (pH 6–8) or weak acidic (pH 4–6) response behaviour have been exploited, relatively less attention is paid to the fluorescent pH probes functioning over extremely acidic range (pH < 4) [20,21]. Thus, it is a great challenge to design new pH indicators with high quantum yield, significant colour change, photo stability and desirable functioning over extremely acidic range (pH < 4).

Indolizines have been found to exhibit a variety of biological activities and have also been investigated for their suitability as dyes for dye sensitized solar cells (DSSC) or organic light emitting devices (OLEDs), owing to their appreciable photo-physical properties [22,23]. However, it is rarely reported that indolizine derivatives are used to make pH fluorescent probes which are suitable for strong acid conditions.

As a continuation of our work on searching for novel fluorescent dyes [24–29], herein, we report an indolizine derivative, 2-acetylindolizine-7-carboxylic acid (**ID-1**), as a new pH probe for acid condition based on intramolecular charge transfer (ICT). Molecules that exhibit ICT are conjugated organic π-systems with acceptor (A) and donor (D) subunits. The photoexcitation of D-A molecules is followed by an electron transfer from donor to acceptor. This process is widely used in the design of ratiometric fluorescent sensors [30–39]. In **ID-1**, acetyl group acts as an acceptor while the carboxyl anion acts as an electron donor to form push-pull system. Compared with other reported pH probes [1,2,40–54], this

* Corresponding author.
E-mail address: xiaoquncao@126.com (X. Cao).

http://dx.doi.org/10.1016/j.snb.2017.02.157
0925-4005/



Scheme 1. Synthesis of sensor **ID-1.**

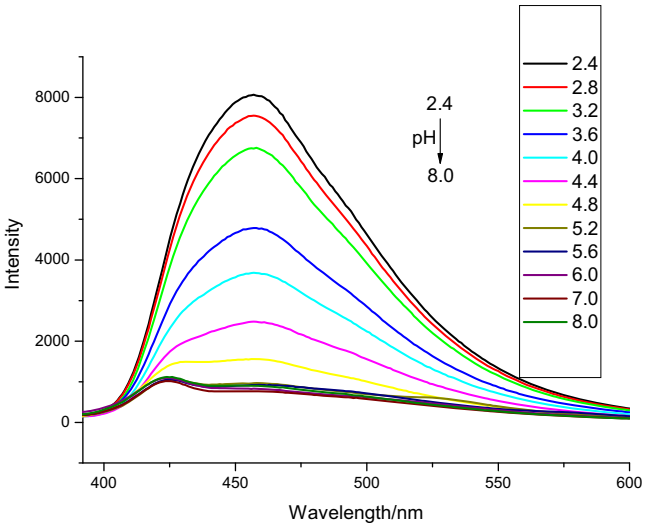


Fig. 1. Fluorescence spectra of the probe **ID-1** (10 μM) in solution (8/2, B-R/DMSO, v/v) with different pH, λ_{ex} = 380 nm.

probe can measure pH values with high quantum yield (φ = 0.52) and high sensitivity in short time (less than 1 min). Furthermore, we have demonstrated the value of this probe by fluorescence imaging in RAW 264.7 cells.

2. Experimental section

2.1. Materials

All reagents and solvents were purchased from commercial sources and used without further purification. The solutions of metal ions were prepared from chlorinated salts which were dissolved in deionized water. Deionized water was used throughout the process of absorption and fluorescence determination. All samples were prepared at room temperature, shaken for 10 s and rested for 10 min before UV–vis and fluorescence determination. Britton-Robinson (B-R) buffer was prepared with 40 mM acetic, boric acid, and phosphoric acid. Dilute hydrochloric acid or sodium hydroxide was used for tuning pH values.

2.2. Instruments

^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Avance 400 (400 MHz) spectrometer using DMSO-d_6 as solvent and tetramethylsilane (TMS) as an internal standard. MS spectra were recorded on a 5800 Matrix Assisted Laser Desorption Ionization-Time of Flight Mass spectrometer (AB SCIEX, U.S.A). Fluorescence measurements were recorded on an F-4600 luminescence spectrophotometer (Hitachi) and UV–vis spectra were recorded on a U-3900 UV–vis Spectrometer (Hitachi). The pH values were measured by the use of a PHSJ-3Fdigital pH-meter (LeiCi, Shanghai).

2.3. Cell culture and imaging

RAW 264.7 cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37 °C in a 5% CO_2/95% air incubator. For living cells imaging experiments, the growth medium was removed and replaced with DMEM without FBS. The cells were treated and incubated with 10 μM of **ID-1** at 37 °C under 5% CO_2 for 10 min. The cells were washed three times with PBS and then cell images were obtained using a confocal microscope from C2+ (Nikon) at excitation of 405 nm.

2.4. Synthesis of probe *ID-1*

Compound ethyl (*E*)-4-bromobut-2-enoate was achieved commercially. Compound **1** and **2** were synthesized as described in the literature [22].

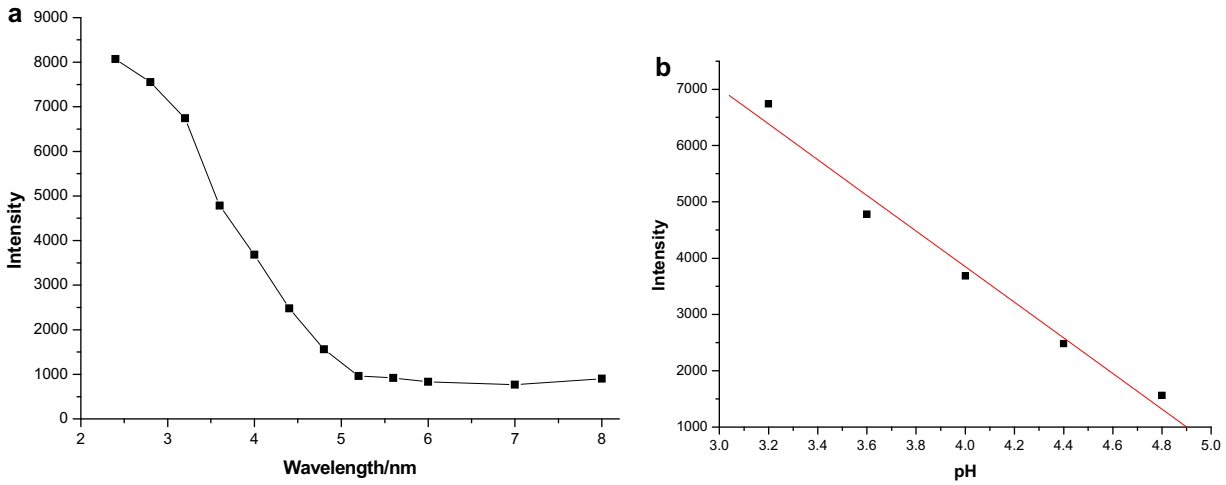


Fig. 2. (a) Fluorescence intensity at 458 nm by pH values according to the fluorescence titration (pH 2.4-8.0). (b) The linear relationship of fluorescence intensity at 455 nm and pH values from 4.4 to 6.0 (R^2 = −0.990).